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ORIGINAL ARTICLE

Immunohistochemical Expression of Gata3 Gene in Patients with Breast Cancer

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ABSTRACT

Breast cancer (BC) is one of the most common cancers in the world. In numerous tissues, including the breast, GATA3 plays an important role in stimulating proliferation and differentiation. The main aims of this study is determining the types of BC (IDC and ILC) then the estimation of the role of GATA3 protein expression by immunohistochemical staining method (IHC) in BC patients and control groups as a biomarker.

The present study was done during the period from October 2020 to April 2021. Sixty seven tissue samples block embedded in wax taken from BC female patients and thirty four of normal non-tumoral breast tissue as a control group collected randomly with their data from three private pathological clinics, these blocks have been prepared between (2014 – 2021), three pathologists re-evaluate each pathologic material.

Regarding to IHC GATA3 protein expression, after histological re-evaluation of slides, the rate of IDC was 80.6% (54 patients) and of ILC was (19.4%) (13 patients).The scoring system +1 (37.3%) and +2(19.4%) increased significantly in BC patients than control (P=0.001), in addition to, the nuclear positive expression of GATA3 decrease significantly in BC patients than control (Odd ratio 2.55, 95% CI 1.45-2.37, P=0.0001), On the other hand, the positivity of GATA3 protein increased significantly in patients with invasive ductal carcinoma (IDC) (P=0.001).

Keyword: GATA3, Breast Cancer, Immunohistochemical Expression.

INTRODUCTION

BC is the most frequently diagnosed type of cancer in women and the second greatest cause of cancer death (Nam *et al.*, 2010) until 2020, BC became the first greteat reason of death in 2020 (WHO fact sheet, 2021). It has the greatest reason of death rate caused by carcinoma (15%), behind lung carcinoma (Baade, 2017), accounting for 458 000 fatalities annually (Altobelli, 2017). BC is a heterogeneous collection of diseases and not a single disease. It can be classified into at least 4 molecular subtypes which included from luminal A, luminal B, basal-like, and HER2-enriched (Pandit *et al.* 2020).

Approximately 70% of BC are positive estrogen receptor (ER)/ positive progesterone receptor (PR) are responded to hormonal therapy whereas the rest 30% of BC was no responding for hormonal therapy. Additional markers can be used to enhance the predictive response of hormone might significantly progress the management proposal for a large number of BC patients (Purvi *et al.*, 2005).

GATA3, also known as a Trans-Acting T-cell-Specific Transcription Factor, is a zinc finger transcription element that governs the differentiation and morphogenesis of a kinds of tissues, involving the breast (Cakir *et al.*, 2017). It has been demonstrated that requirement of GATA3 is essential for the development of luminal cells in mammary glands (Fu *et al.*, 2020).

The expression of the GATA3 protein is associated with hormone responsiveness and ER expression, showing that it plays a role in the carcinogenesis of ER-positive BC (Hruschka *et al.*, 2020). A high level of GATA3 expression is related to a low grade of tumor and a sluggish ratio of proliferation. The highest amount of GATA3 expression is observed in tumors classified as 'luminal A,' which is the subtype related to the best survival consequence. Therefore, the level of GATA3 expression may be predictive of patient outcome, implying that it may be useful as a prognostic indicator in BC (Perou *et al.*, 2000; Demir *et al.*, 2010).This is to be anticipated, considering that ER-positive breast tumors are typically well-differentiated morphologically and have a favorable prognosis(Shaoxian *et al.*, 2016). About 96 percent of ER -positive breast tumors showed GATA3, whereas only 22% of ER -negative tumors do.

Aim of the study: is studying the expression of GATA3 gene in patients with breast cancer by estimation of GATA3 protein expression in BC patients and control groups by the IHC.

Collection of Samples: This retrospective study was accomplished in Middle Euphrates Unit for Cancer Research, Faculty of Medicine, University of Kufa, during the period from October 2020 to April 2021.Immunohistochemistry study were completed in Middle Euphrates Unit for Cancer Research, Faculty of Medicine, University of Kufa. It is carried out on sixty-seven cases of BC in the form of an available Paraffin blocks who underwent surgical resection between 2015 and 2020, these data collected from the archives of three private laboratories. Three pathologists histologically re-evaluated each pathologic material, Patients clinicopathological characteristics are obtained from electronic medical records of these laboratories. GATA3 expression is achieved by IHC. In addition, thirty-four blocks of normal non-tumor breast tissue are collected randomly during the collection of breast cancer.

Histopathological Examination: Five µm-thick layers are cut from paraffin embeded tissues. These sections stained by using routine stain method called Haematoxylin and Eosin method (Falkeholm et al.,2001).

Statistical analysis: Data have been analysed by using SPSS version 21 and Pad Graphprism version 7, nominal variables were expressed as number and percent. Student t-test was used to compare mean between two groups,

when variables were normally distributed. Chi-square test was used to compare frequencies. P-value was regarded as significant when it was less than or equal to 0.05.

RESULTS:

The patients group was subdivided histologically into two subgroups; Invasive Ductal Carcinoma (IDC) and Invasive Lobular Carcinoma (ILC). Majority of cases 54 cases (80.6%) were of IDC subtype whereas ILC subtype cases were seen in 13 (19.4%) cases, as seen in (Figure 1).Nuclear IHC positive expression of GATA3 was decreased significantly in patients group 42 patients (62.7%) in comparison for control group 34 persons (100%) while the negative expression of GATA3 was decreased significantly in patients group (25 patients) (37.3%) as compared with control group 0 persons (0.0%) (P=0.0001). On the other hand, Odd ratio for this protein was 2.55 95% CI (1.45-2.37) as shown in (Table 1) and (Figures 1,2, and 3).

Nuclear IHC of GATA3 expression	Control Group		Patients Group		Odd ratio (95% CI)
	No.	%	No.	%	
Positive expression	34	100.00	42	62.7	2.55 -1.45) (2.37
Negative expression	0	%0.0	25	37.3	
Total	34	100.00	67	100.0 0	

Table 1: GATA3 nuclear expression in two groups of the study.
P=0.0001
CI = Confidence interval

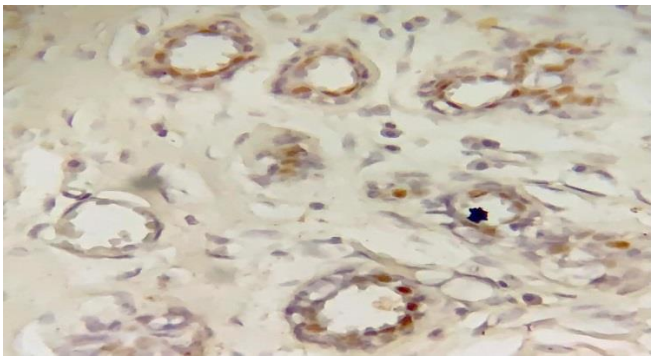

Figure 1: Normal breast acini tissue positive for GATA3 IHC 400X.

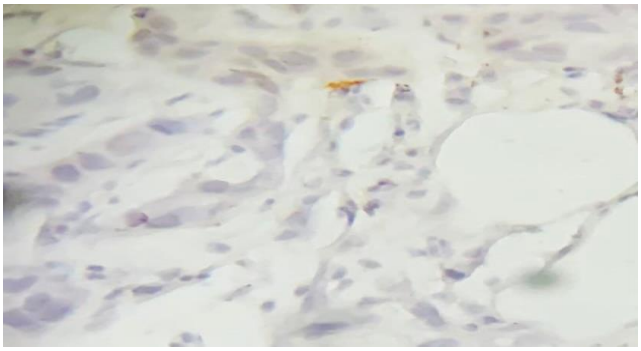

Figure 2: Invasive lobular carcinoma negative for GATA3 IHC 400X.

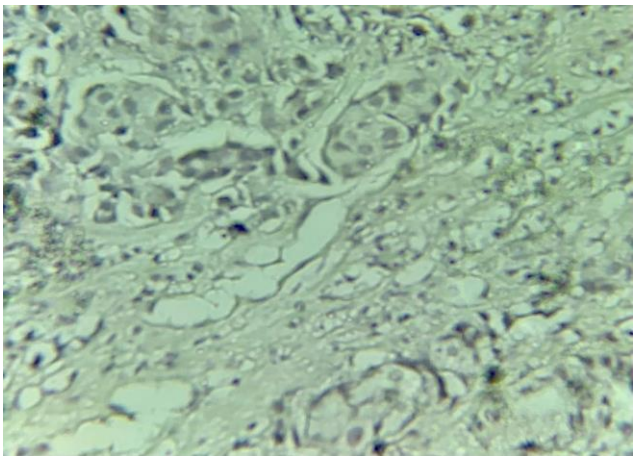

Figure 3: Moderately differentiated invasive ductal carcinoma of breast negative for GATA3 IHC x400.

GATA3 protein expression was increased significantly in patients group more than in control group (P=0.001).Twenty-five patients (19.4%) have score +1 expression, ten patients (14.9%) have score +2 patients, eleven patients (16.4%) have score +3 expression and eight patients (11.9%) have score +4 expression. All control subjects were positive for GATA3 expression, fourteen patients (41.2%) and fourteen patients (41.2%) were +3 and +4 respectively as seen in (Table 2) and (Figure 4).

Scoring	Control		Patients		Total	
	No.	%	No.	%	No.	%
0	0	%0.0	25	%37.3	25	%24.8
1+	0	%0.0	13	%19.4	13	%12.9
2+	6	%17.6	10	%14.9	16	%15.8
3+	14	%41.2	11	%16.4	25	%24.8
4+	14	%41.2	8	%11.9	22	%21.8

Table 2: GATA3 protein expression in BC in two groups scoring.
P=0.001

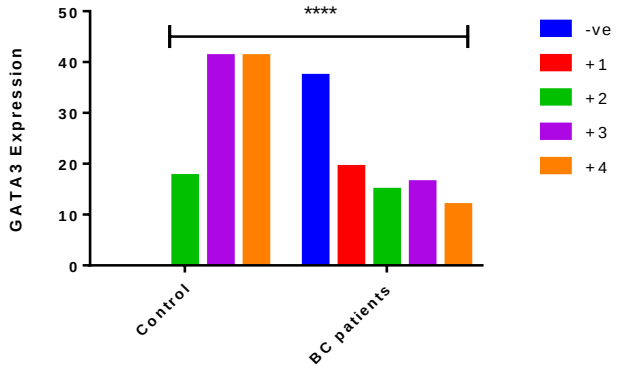

Figure 4: GATA3 protein expression in BC in two groups scoring.

**** = (P=0.001)

Nuclear positive expression of GATA3 was increased significantly in patients with (IDC) 39 patients (72.2%) when compared with (ILC) patients individuals (32.1 %%) (P=0.001) as seen in (Table 3) and (Figure 5).

Table 3: BC Histological Types and GATA3 protein expression.

Histological Type of BC	Negative expression		Positive expression		Total	
	No.	%	No.	%	No.	%
Invasive ductal carcinoma	15	%27.8	39	%72.2	54	100 %
Invasive lobular carcinoma	10	%76.9	3	%32.1	13	100 %

P=0.001, Pearson Chi-Square Test

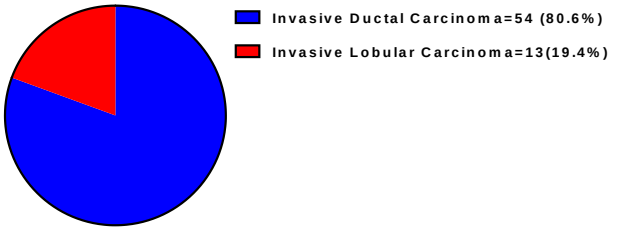

Figure 5: Histological subtypes of BC.

DISCUSSION:

This study discovered that positive expression of GATA3 protein decreased significantly in the tissues of BC patients compared to controls with normal tissue that contains no negative GATA3 expression, whereas 37.3 percent of BC patients enrolled in this study have negative GATA3 expression. In agree with the current outcomes, preceding study has proven that the GATA3 expression level in patients of BC is reduced in comparison with normal tissue of breast in control group (Hisamatsu *et al.*, 2015; Thakkar *et al*., 2015; Guo *et al.*, 2017; Yang *et al*., 2017; Abd-Elghany *et al.*, 2019). Luminal subtypes are the most prevalent type of breast cancer and exhibit the greatest levels of GATA3 expression (Mehra *et al*., 2005; Voduc *et al.*, 2008). GATA3 levels have been demonstrated to alter the ability of luminal stem/progenitor cells to initiate tumors but not basal stem/progenitor cells, showing a GATA3 vital action for in adjusting the distinction of the cells of luminal progenitor in normal state or malignant tissue of BC (Asselin-Labat *et al*., 2011). GATA3 is frequently co-expressed with its exact aim, the receptor of estrogen (ER), and functions in a positive feedback circle (Albergaria *et al.*, 2009; Ciocca *et al*., 2009).

GATA3 is a much characterized reason in the formation of glandular cells of breast. GATA3 is required for development of mammary during embryonic life and is implicated in the maintenance of the differentiated condition of the mammary gland's luminal epithelial cells in adults (Yoon *et al.*, 2010; Tokuda *et al.*, 2012). Not amazingly, expression of GATA3 loss has been implicated in the pathophysiology of BC, with lesser expression degrees being associated with receptors of progesterone and estrogen (ER and PR) negative, overexpression of Her2/neu, and bad prognosis (Ademuyiwa *et al.*, 2010; Naoi *et al.*, 2011; Oshima *et al.*, 2011). Overexpression of GATA3 is believed to cause the abnormal production of aromatase in BC (Brychtova *et al.*, 2011).

Previously, we observed that GATA3 expression is dramatically enhanced in cases of BC as compared with normal epithelium of breast using expression of gene array analysis and real-time quantitative reverse transcriptase PCR (Ren *et al.*, 2004).

GATA3 is required for appropriate development of mammary gland. GATA3 is demonstrated to stimulate luminal cell development when it was introduced into a pure mammary progenitor-enriched people (Asselin-Labat *et al.*, 2007). GATA3 is existing in cells of the luminal epithelium of human breast tissue as well as is also expressed in BC (Kouros-Meh *et al.*, 2004). In BC, GATA3 expression is intensely correlated with forkhead box A1 (FOXA1) expression and estrogen receptor (ER) (Kouros-Mehr *et al.*, 2008). FOXA1 is required for the transcriptional function of ER (Bernardo et al, 2010). Additionally, it has been reported that GATA3 may act as a mucin1(MUC1) transcriptional upregulation mediator in BC. These findings strongly suggest the requirement of GATA3 for the formation and discrimination of cells of luminal epithelium, which is logical with GATA3 being expressed preferentially in luminal-type breast cancer (Demir *et al.*, 2010).

CONCLUSION:

These findings suggest that GATA3 is involved in epithelial cell growth control and differentiation maintenance, and that GATA3 may play a role in carcinogenesis in ER-positive BC. GATA3 as a more reliable and sensitive diagnostic marker for ductal BC and as distinguishable marker for molecular subtypes of BC.

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